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Genetic Interaction between the β′ Subunit of RNA Polymerase and the Arginine-Rich Domain of *Escherichia coli nusA* Protein

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The *nusA11* mutation causes reduced transcription termination and temperature-sensitive growth of *Escherichia coli*. Suppressor mutations that restored growth of *nusA11* mutant cells were isolated and named *sna* mutations. The intergenic suppressor mutation *sna-10* was located in the *rpoC* gene at 90 min, which encodes the β′ subunit of RNA polymerase. *sna-10* complemented the defect in t_{R1} termination caused by *nusA11* and by itself stimulated termination of transcription at the λ t_{R1} terminator. *sna-10* is specific to the *nusA11* allele and unable to suppress cold-sensitive growth of the *nusA10* mutant. *nusA10* carried two base substitutions at positions 311 and 634, causing two amino acid changes from the wild-type sequence. During these studies, we found three −1 frameshift errors in the wild-type *nusA* sequence; the correct sequence was confirmed by the peptide sequence and gene fusion analyses. The revised sequence revealed that *nusA1* and *nusA11* are located in an arginine-rich peptide region and substitute arginine and aspartate for leucine 183 and glycine 181, respectively. The intragenic suppressor study indicated that the *nusA11* mutation can be suppressed by changing the mutated aspartate 181 to alanine or changing aspartate 84 to tyrosine.

The *nusA* gene of *Escherichia coli* participates in both termination and antitermination of transcription (9, 11, 27, 40). The gene was first identified by isolation of the *nusA1* mutation, which restricts bacteriophage λ growth by preventing the antitermination activity of the λ N protein (7, 8). This *nusA1* mutation nevertheless does not affect bacterial growth and bacterial transcription. Isolation of amber, temperature-sensitive, and cold-sensitive mutations has established that *nusA* function is essential for growth of *E. coli* (37, 41, 46, 51).

The primary defect in the temperature-sensitive *nusA11* mutant is the inability to terminate transcription normally at termination sites (37, 38). In view of the previous finding that the *nusA* gene product plays a positive role in the antitermination of λ transcription by acting as a cofactor of the N protein (for reviews, see references 9 and 11), it was expected that *nusA11* would inhibit or reduce λ phage growth. However, it does not restrict λ growth under permissive or nonpermissive conditions, but rather enhances λ phage growth in *N* mutant conditions (37, 38). This finding was explained by assuming that the λ antitermination reaction is not necessary for phage growth in the *nusA11* mutant simply because the *nusA11* mutant is defective in termination and bypasses the requirement for antitermination. In fact, other conditionally lethal *nusA* mutations, *nusA134* (temperature-sensitive lethal amber mutation) and *nusA10* (cold-sensitive lethal mutation), partially reduce λ *N* antitermination and restrict growth of λ phage variants carrying the IS2 transposon, which encodes multiple termination sites, or the *bio-256* substitution, which produces weak N activity by COOH-terminal substitution (46, 51). In the purified or crude in vitro transcription system, NusA exerts comparable effects. It causes or accentuates pausing of RNA polymerase at specific sites, resulting in the enhancement of termination (6, 16, 24, 25), whereas it stimulates N and Q antitermination (5, 13, 53) or by itself prevents premature rho termination at some sites, giving rise to an increase of readthrough transcription (27, 55). These observations demonstrate the capacity of NusA to affect transcription as a modulator.

NusA protein binds to the core RNA polymerase, presumably after release of σ factor upon transcription initiation, and remains associated with the transcribing complex until termination of transcription (14). NusA also interacts with λ N protein, RNA, *E. coli* rho factor, and perhaps *nusB* protein (10, 15, 18, 47, 50, 52). The pleiotropic roles of NusA during transcription may be attributed to the capacity for multiple interactions with these regulatory components. One can speculate that NusA plays the role of an adaptor to link regulatory factors to RNA polymerase and to induce conformational change in the polymerase which alters transcriptional specificity.

The NusA-RNA polymerase interaction has been supported by genetic studies on *rpoB* mutations, which affect the β subunit of RNA polymerase. Reduced termination of transcription by *nusA11* or *nusA10* is compensated for by rifampin-resistant β subunit mutations to some extent (22), and the *snu* mutation in *rpoB* accentuates the inhibitory effect of *nusA1* on λ N antitermination (3). These results indicate functional interaction between RNA polymerase and NusA protein. However, it has not been discovered whether this functional interaction is based on the direct binding of NusA to the β subunit or through binding to some other subunit(s).

In spite of the transcription studies, little is known about the structural and functional organization of the NusA protein. The primary sequence of NusA has been deduced by nucleotide sequence analyses (21). We have defined the base substitutions caused by *nusA1* and *nusA11* at positions 548 and 542, respectively (44). However, these base substitutions had been misdecoded because of frameshift sequence errors in the wild-type *nusA* gene, as shown in this and an accompanying article (4a). We ensured the accuracy of the revised part of the *nusA* sequence by peptide and gene fusion analyses. In the affected sequence, the *nusA1* and *nusA11* mutations are located in an arginine-rich peptide region. It is

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TABLE 1. *E. coli* K-12 strains

Strain	Description[a]	Source or reference
KY1092	*argG metB trpE9829*(Am) *tyr*(Am) *sup-126*	37
KY2572	*his ilv trpE9829*(Am) *lacZy14*(Am) *tonA*(Am) Tet^r (*wxy*::Tn*10*) *tyr*(Am) *tsx*(Am) *btuB sup-126*	This study
YN2351	Same as KY1092 but *argG*$^+$ *nusA11*(Ts)	37
YN3428	Same as YN2351 but *sna-10*	This study
RM37	Same as YN3428 but λ *papa* lysogen	This study
YN2634	Same as YN3428 but Tet^r (*wxy*::Tn*10*)	This study
RM24	YN3428 Rif^r (*rpoB*)	This study
YN2345	Same as KY1092 but Tet^r (*wxy*::Tn*10*)	This study
KY1397	Hfr (O *ilv thr gal*) *thy btuB*	Lab strain
R594	*gal-1 gal-2 lac rpsL sup*^{0}	4
RM63	Same as R594 but *sna-10* Tet^r (*wxy*::Tn*10*)	This study
RM61	Same as R594 but *nusA1*	This study
RM64	Same as R594 but *nusA1 sna-10* Tet^r (*wxy*::Tn*10*)	This study
RM62	Same as R594 but *nusA11*	This study
RM65	Same as R594 but *nusA11 sna-10* Tet^r (*wxy*::Tn*10*)	This study
RM35	Same as R594 but *argG*::Tn*5 nusA10*(Cs)	This study
RM38	Same as R594 but *argG*::Tn*5 nusA10*(Cs) *sna-10* Tet^r (*wxy*:Tn*10*)	This study
CAG3864	*nusA10*(Cs) *argG*::Tn*5 btuB*::Tn*10 galK2 rpsL200*	C. A. Gross
RM18	Same as CAG3864 but *argG*$^+$	This study
AT1103	Same as R594 but *nusA134*(Am) Tet^r (*wxz*::Tn*10*)	51
YN2458	Same as R594 but *nusA11* Tet^r (*wxz*::Tn*10*)	41
TAP112	*his ilv rpsL galK*(Am) *bioA* (λ {Δ[*int-ral*] *N*::Tn*5* c1857 Δ[*cro-bioA*]}	D. L. Court
TAP203	Same as TAP112 but *hip*::Cm^r *himA*Δ81 *pkfB*::Tn*10*	D. L. Court

[a] Genetic symbols are those described by Bachmann (2). All strains were F$^-$ except KY1397. Strains listed as "This study" were constructed by P1 transduction of relevant markers with Tn*10*, Tn*5*, and *argG*$^+$. Phenotypes of *nusA* mutations were scored by growth at high and low temperatures or by sensitivity to phages λ *papa* and λ *nin5*. RM24 was a spontaneous rifampin-resistant isolate from YN3428.

intriguing that the arginine-rich peptide motif is conserved in several RNA-binding proteins, including λ N protein, and is likely to play a crucial role in recognition of RNA signals (30), implying the importance of the arginine-rich domain of NusA.

This work describes the isolation of suppressor mutations of *nusA11* and was undertaken in order to investigate the structure-function relationships of NusA and to identify *E. coli* regulatory factors which interact with NusA protein in cellular termination and antitermination.

MATERIALS AND METHODS

Bacterial and phage strains. All bacterial strains used are listed in Table 1. Phage P1 *vir* was used for transductional mapping. Other phages used were λ *papa*, λ *nin5*, λ EMBL4 (12), λ *rif*^{d}18 (26), and λ NM540*c* (a clear-plaque derivative of λ NM540 constructed in this study; see reference 35).

Media and antibody. Minimal medium was medium E or M56 (34) with appropriate supplements. YT broth contained 0.1% (wt/vol) yeast extract, 1% (wt/vol) tryptone (Difco Laboratories, Detroit, Mich.), and 0.25% (wt/vol) NaCl. Anti-NusA rabbit antiserum and the murine monoclonal antibody N14 were described previously (39).

Plasmids. The cold-sensitive (Cs) *nusA10* mutant DNA was isolated from RM18, an Arg$^+$ derivative of CAG3864 (*argG*::Tn*10 nusA10*). The chromosomal DNA was digested with *Eco*RI and ligated into the same site of λ EMBL4. The phage library was screened by plaque hybridization with the wild-type *nusA* DNA probe, and the transducing phage λ EMBL4-Cs10-3, carrying the 16-kb *argG-nusA* fragment, was isolated. The 8-kb *Sal*I-*Hin*dIII fragment of the insert was recloned into the plasmid vectors pBR322 and pACYC184, giving rise to pBR-Cs10-3 and pAC-Cs10-3, respectively, and subjected to DNA sequence analysis. The wild-type *rpoC* DNA was subcloned from the transducing phage λ *rif*^{d}18. The 10.5-kb *Hin*dIII DNA encoding *rplL-rpoB-rpoC* was isolated from λ *rif*^{d}18 DNA and ligated into the *Hin*dIII site of plasmid pSU2719 (a multi-cloning-site derivative of vector pACYC184 [32]), generating the chimeric plasmid pSU-LBC^W. Then, internal *Bgl*II fragments in the *rpoC* gene were removed from the plasmid pSU-LBC^W to make plasmid pSU-LB^W; pSU-LB^W encodes *rplL* and *rpoB*. The *rpoB* and *rpoC* genes in plasmid pSU-LBC^W were disrupted by insertion of the 1.3-kb Kan^r gene segment at *Cla*I sites to form plasmids pSU-L(B::Kan)C^W and pSU-LB(C::Kan)^W, respectively.

The *sna-10* mutant DNA library was constructed by ligating *Hin*dIII digests of bacterial DNA to the phage vector λ NM540*c*. The mutated *rpoC* gene was cloned from this library by plaque hybridization with the wild-type *rplL-rpoB-rpoC* DNA probe. The resulting transducing phage, λ NM540*c-sna-10*, carried the 10.5-kb *Hin*dIII fragment. This insert was recloned into pSU2719, giving rise to pSU-LBC^M. (The *Bgl*II fragments encoding the COOH-terminal part of *rpoC* were removed from plasmid pSU-LBC^M to form pSU-LB^M, which was structurally equivalent to pSU-LB^W.) The 5.4-kb *Sph*I-*Hin*dIII fragment, which encodes the mutant *rpoC* gene alone, was recloned in pSU-2719 to form pSU-C^M.

Plasmids pYN87 (*nusA*$^+$), pMS1 (*nusA11*), and pYN85N (*nusA1*) are pBR322 derivatives carrying the 5.3-kb *Sal*I-*Bgl*II fragment encoding truncated NusA, which contains wild-type and mutated *nusA* alleles (28, 39). pKG100 is a terminator-cloning plasmid vector (33), and pMZ105 carries a λ t_{R1} terminator insert in plasmid pKG100 (37). The in-frame fusion vector pWS50 (48) and its derivatives containing truncated *nusA* fragments, pWS50-12, -30, -37, -38, and -56, were described previously (39).

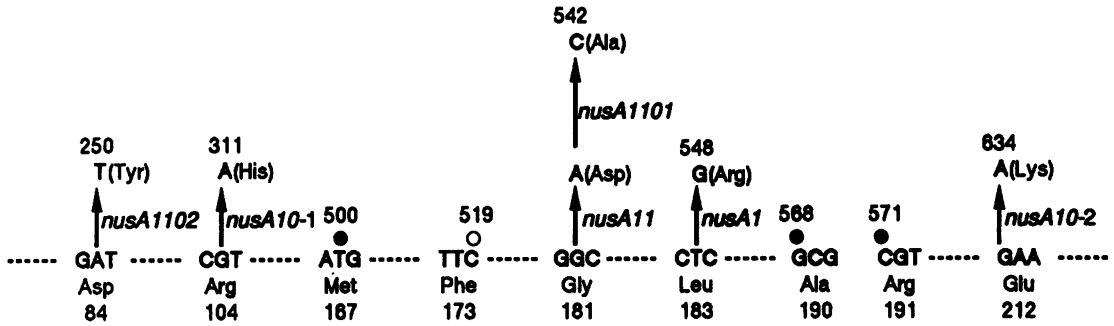

FIG. 1. Nucleotide sequence corrections and substitutions caused by *nusA* mutations. Nucleotide and amino acid positions are counted from the translation start site of *nusA*. An open circle represents a position of misread sequence, and solid circles represent deletion errors in the previous sequence (21, 45).

Assay of galactokinase activity. Cells carrying plasmid pBR322, pKG100, or pMZ105 were grown in M56 medium supplemented with 0.2% (wt/vol) fructose as a carbon source, 0.2% casamino acids (Difco), 10 μg of thiamine per ml, 3 μg of biotin per ml, 1 mM magnesium sulfate, and 50 μg of ampicillin per ml. Cultures of these cells growing exponentially at 32°C (for *nusA11* and *nusA1*) or 42°C (for *nusA10*) and cultures that had been exposed to nonpermissive temperatures were lysed by vigorous shaking with toluene. Galactokinase activities were assayed as described by Adhya and Miller (1).

DNA sequence analysis. Cold-sensitive *nusA10* mutant DNA cloned in pBR-Cs10-3 or pAC-Cs10-3 was sequenced by the double-stranded DNA dideoxy chain termination method described by Sanger et al. (45) and Maniatis et al. (31) with appropriate 17- or 20-mer primers, which enabled us to sequence the entire coding region.

Peptide analysis. NusA protein was purified from overproducing cells (YN2734 [36]) to homogeneity as described previously (50). The purified protein (~1 mg) was digested either with *Achromobacter* protease I (lysyl endopeptidase; Wako Pure Chemical Industry, Osaka, Japan) in 50 mM Tris-HCl (pH 9.0) at 37°C for 20 h or with cyanogen bromide in 70% formic acid at 37°C for 25 h. One half of the protease digests was fractionated by high-performance liquid chromatography (HPLC) with an ODS-120T column (Toyo Soda Co., Tokyo, Japan) and subjected to amino sequence analyses with an automatic protein sequencer (Applied Biosystems, California). The other half was applied to a column (approximately 0.6 ml) of Sepharose 4B conjugated with monoclonal antibody N14, which was presumed to recognize a polypeptide region of *nusA1* and *nusA11* mutation sites (39). The peptide bound to the column was eluted with aqueous acetic acid (pH 2.5). The eluate was evaporated in vacuo to dryness. The cyanogen bromide peptide purified by the affinity column was analyzed directly for the amino acid sequence, and the affinity-purified lysyl endopeptidase fragment was further digested with *Staphylococcus aureus* V8 protease (Wako Pure Chemical Industry) in 50 mM ammonium bicarbonate (pH 7.8). The hydrolysates were fractionated by HPLC, and the peptides isolated were analyzed for amino acid sequence. In addition to these endopeptidase and cyanogen bromide digests, undigested NusA protein was sequenced directly.

Other methods. Immunoblotting analysis was done with anti-NusA rabbit antiserum and the murine monoclonal antibody N14 as described previously (39). Southern hybridization analysis was conducted as described previously (31). Electrophoresis of proteins was done as described by Laemmli (29).

RESULTS

Arginine-rich domain of NusA in the revised sequence. In spite of the previous effort (44) to correct errors in the original nucleotide sequence of *nusA* published by Ishii et al. (21), four errors were found during the course of the sequence study of *nusA11* suppressor mutations and the cold-sensitive mutation *nusA10*. The nucleotide C had been misread as T at position 519, and three nucleotides had been omitted at position 500 (T insert), 568 (G insert), and 571 (C insert), which caused a reading frame shift between codon positions 167 and 191 (Fig. 1 and 2). As the *nusA11* and *nusA1* mutations replaced nucleotides at base positions 542 and 548, respectively, the mutant amino acid substitutions were misinterpreted. The revised *nusA* sequence is shown in Fig. 1.

In-frame *nusA-lacZ* fusion peptides were analyzed to test the reading frame of *nusA*. Plasmid pWS50 is a vector designed for selective cloning and expression of an open reading frame by protein fusion (48). The cloning site of pWS50 is an *Nru*I site which is between an out-of-frame fusion of the λ cII and *E. coli lacZ* genes. Cleavage by *Nru*I leaves one base of a codon at both ends, and insertion of an

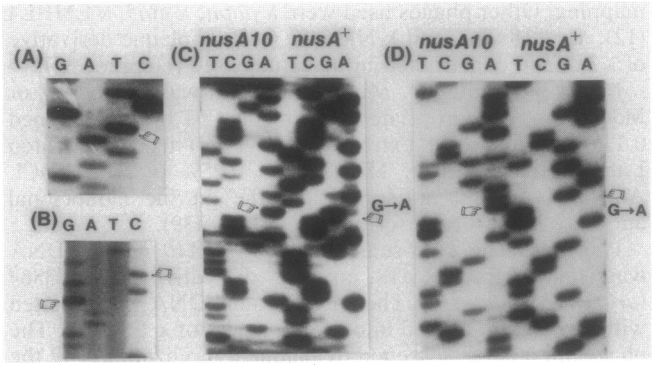

FIG. 2. DNA sequence of *nusA* containing revised positions and base substitutions found in the cold-sensitive *nusA10* mutant. (A) Correction of base position 500; presence of T. (B) Correction of base positions 568 and 571; presence of G and C, respectively. (C) Base substitution in *nusA10*; a G-to-A change at position 311. (D) Base substitution in *nusA10*; a G-to-A change at position 634. Corresponding bases are marked.

TABLE 2. Fusion proteins containing NusA peptides

Fusion plasmid	Nucleotides[a]	Nucleotide sequence[b]		Amino acids[c]
		cII junction	lacZ junction	
pWS50-12	370–710	G GCG ATG	ATC GCC	(Ala-124–Ala-237*)
pWS50-30	467–677	GAC AAC	GCA GCC	Asn-157–Ala-226*
pWS50-37	512–1085	GAA AAC	AAA TAC	Glu-171*–Tyr-362*
pWS50-38	548–674	GTC TAT	AAA GCC	Tyr-184–Ala-225*
pWS50-56	985–1241	G TCG CAG	GCA CTC	(Ser-329–Leu-414*)

[a] cII-*nusA* and *nusA-lacZ* junctions were determined by nucleotide sequence analysis with cII and *lacZ* primers.

[b] Sequences of the 5′ and 3′ junctions of the *nusA* fragment are indicated. The *nusA* sequences presented in in-frame triplets according to the revised sequence are underlined. The 5′ and 3′ terminal nucleotides (G and C) that are not underlined correspond to cII and *lacZ* ends, respectively.

[c] Peptide regions of *nusA* expressed in fusion proteins are presented. Parentheses indicate that the NH_2-terminal amino acid of the *nusA* peptide was not determined; *nusA-lacZ* fusion peptides may be synthesized presumably by internal translation start within the *nusA* insert. Asterisks denote that the terminal two nucleotides of *nusA* inserts regenerated the same amino acid by ligation into the *Nru*I site of pWS50. We have reported epitope mapping of murine monoclonal anti-NusA antibodies with these fusion proteins (39). The reactivities of these *nusA* fusion proteins to antibodies are the same as published: namely, antibody N14 reacts with fusions pWS50-12, -30, and -37 but not with -38 and -56; N2 reacts only with pWS50-56; the other five antibodies (N1, N8, N9, N17, and N18) react with all fusions except pWS50-56. The epitope region can be slightly modified from the previous one (39) according to the revised peptide sequence presented in this column.

open reading frame with two bases at both ends is able to generate a cII-insert-*lacZ* in-frame fusion polypeptide.

Fusion plasmids pWS50-12 through pWS50-56 are derivatives of pWS50 which contain fragments of *nusA*. These fusions were selected as $LacZ^+$ and reactive to anti-NusA rabbit antiserum (39). Therefore, there is no doubt that parts of the NusA polypeptide were fused to β-galactosidase and synthesized from these plasmids. The sequences of the fusion point in the plasmids are shown in Table 2. All of the *nusA-lacZ* junctions were in frame. On the other hand, the cII-*nusA* junctions in pWS50-30, -37, and -38 were in frame according to the revised sequence, whereas those in pWS50-12 and -56 were out of frame. The latter two out-of-frame fusions can be explained by assuming an alternative translation start in the *nusA* segment or, though less likely, occurrence of mutations in the fusion constructs which coordinate the coding frame to synthesize cII-*nusA-lacZ* fusion proteins.

To test the above possibilities, expression of fusion proteins from these plasmids was examined in IHF^+ (TAP112) and IHF^- (TAP203) cells. IHF is composed of the *himA* and *hip-himD* gene products and is required for good cII expression (19, 42). Cells carrying the fusion plasmids were grown at 32°C, shifted to 42°C, and pulse-labeled with [^{35}S]methionine; the labeled proteins were analyzed by electrophoresis. Synthesis of fusion proteins from pWS50-30, -37, and -38 was markedly reduced in IHF^- cells, whereas synthesis from pWS50-12 and -56 was not affected by a null IHF mutation (data not shown). This suggested that the former in-frame fusions are synthesized from the cII start site, while the latter out-of-frame fusions are synthesized from an internal translation start, IHF independent, presumably in the *nusA* segment.

Finally, we analyzed the peptide sequence of NusA. Purified NusA protein was digested enzymatically by lysyl endopeptidase and protease V8 or chemically by cyanogen bromide. These digests were fractionated by HPLC or purified by affinity chromatography with a Sepharose 4B column conjugated with the monoclonal antibody N14, which by mutation analysis recognizes glycine 181 and leucine 183 (39). Thirteen peptides and undigested NusA protein were subjected to NH_2-terminal amino acid sequence analyses with an automatic protein sequencer (Table 3). One hundred forty-eight of the 495 NusA amino acids were determined. The peptide sequences are consistent with those predicted by the revised nucleotide sequence of the *nusA* gene (Fig. 3), in particular, the peptide sequence between positions 160 and 222, which included wild-type alleles of *nusA1* and *nusA11* as well as the four nucleotides misread previously.

Accordingly, part of the *nusA* sequence was revised on the basis of the nucleotide analysis, gene fusion study, and peptide sequence analysis. The revised molecular mass of NusA is 54,981 Da, comprising 495 amino acids. The correct amino acid substitutions caused by the *nusA11* and *nusA1* mutations are a glycine-to-aspartate change at position 181 and a leucine-to-arginine change at position 183, respectively. These residues are located in an arginine-rich peptide domain of NusA; of 33 total arginine residues, seven are clustered in the 164 to 191 region.

TABLE 3. Amino acid sequence of NusA peptides generated by lysyl endopeptidase, protease V8, and cyanogen bromide digestion

Peptide no.	Amino acid sequence	Corresponding positions
1	MNKEILAVVEAVSNEKALP[a]	1–19
2	IFEALESALATATK[b]	23–36
3	AVILRE[c]	160–165
4	DMLPRE[c]	166–171
5	LPRENFRPGDRVRGVLYS[d]	168–185
6	YSVRPE[c]	184–189
7	ARGAQLFVTRSK[c]	190–201
8	FVTRSK[c]	196–201
9	PEMLIELFRIEVPEIGEEV[b]	202–220
10	LIELF[e]	205–209
11	IGEEVIE[f]	216–222
12	PGSRAKIAVKTNDKRI[e]	230–245
13	YLDIDEDFATVLVEEGFST[b]	362–380
14	PADDLLNLEGVDRDLAF[b]	430–446

[a] Intact NusA protein was directly sequenced.

[b] Lysyl endopeptidase digests of NusA were fractionated by HPLC and sequenced.

[c] Lysyl endopeptidase digests of NusA were purified by affinity chromatography with an N14 antibody column. The purified peptide was further cleaved by protease V8, and the resulting hydrolysates were fractionated by HPLC and sequenced.

[d] Cyanogen bromide digests of NusA were purified by affinity chromatography with the N14 antibody column, and the purified peptide was directly sequenced.

[e] Cyanogen bromide digests of NusA were fractionated by HPLC and sequenced.

[f] Lysyl endopeptidase and protease V8 double digests of NusA were fractionated by HPLC and sequenced.

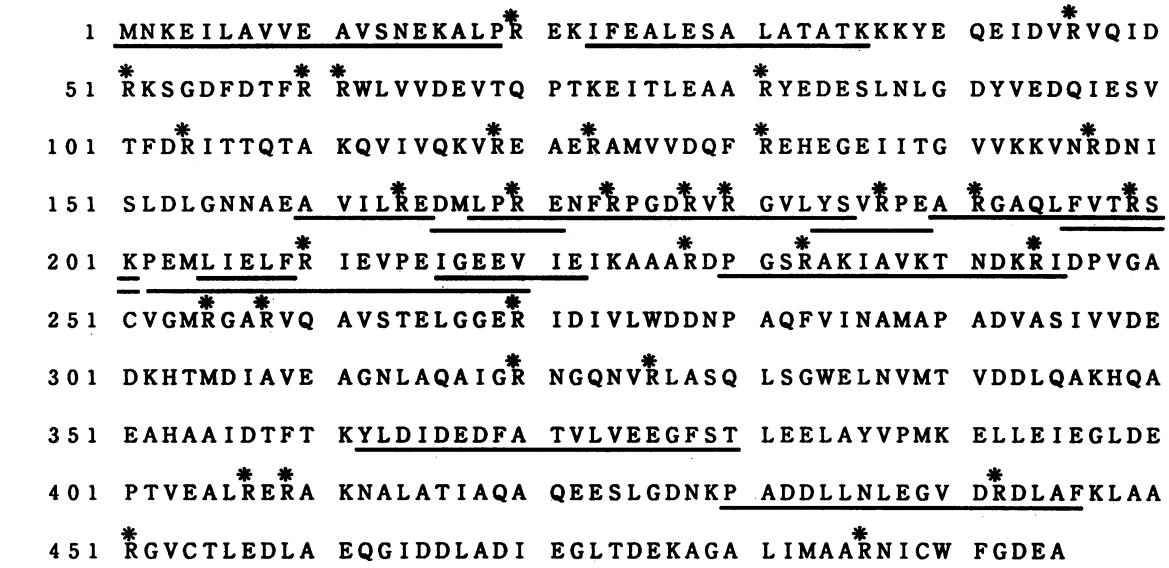

FIG. 3. Amino acid sequence of NusA presented in the single-letter code. Sequences determined by peptide sequencing analysis (Table 3) are underlined. Asterisks indicate arginine (R) residues.

Sequence analysis of cold-sensitive *nusA10*. *nusA10* was isolated as a conditionally lethal cold-sensitive mutation. It has been shown by complementation and genetic crosses that *nusA10* is in the same gene as *nusA1* (46). We cloned the *nusA10* allele into λ EMBL4 and plasmid vectors and sequenced the entire gene for a mutational change. The data indicated that two bases were substituted in *nusA*, a G-to-A change at position 311 and a G-to-A change at position 634 (Fig. 2). Thus, arginine 104 was replaced by histidine and glutamate 212 was replaced by lysine. These base changes were tentatively designated *nusA10-1* and *nusA10-2*, respectively. The same substitutions were found by Craven and Friedman (4a). *nusA10-1* was a basic-to-basic amino acid change, while *nusA10-2* was an acidic-to-basic change. G-to-A substitutions are consistent with the fact that hydroxylamine was used as a mutagen. It is not known whether both changes are required for the cold-sensitive phenotype. However, it is intriguing that the original *nusA10* mutant strain did not grow at 31°C on YT plates but produced revertant colonies at 31°C at high frequency. This might be interpreted as indicating that both changes are needed for the strict cold-sensitive lethality and that reversion or suppressor mutation of either substitution leads to leaky cold-sensitive growth at high frequency.

Growth of the *nusA10* strain at 25°C was restored upon transformation with pBR322 or pACYC184 plasmids carrying the wild-type *nusA* gene. However, the wild-type *E. coli* strain R594 grew slowly at 25°C upon transformation with pBR-Cs10-3 or pAC-Cs10-3 carrying the *nusA10* allele (data not shown). The frequency of transformation of *nusA11* and *nusA1* mutant cells with the plasmid encoding *nusA10* was markedly low (data not shown). These observations suggest some interference effect of overproduced NusA10 protein.

Intragenic *nusA11* suppressor mutations. Suppressor or reversion mutations of *nusA11* were isolated as temperature-resistant colonies, selected at 42°C from YN2458 (*nusA11*) cells carrying pMS1, which encodes the *nusA11* mutant protein, on YT plates containing ampicillin. The population of temperature-resistant colonies was mixed, and plasmid DNAs were isolated as mixtures and used to retransform YN2458 cells. The resulting transformant colonies which grew at 42°C were expected to contain pMS1 derivatives whose *nusA11* allele had reverted to wild type or was suppressed by another mutation in the same plasmid. Plasmid DNAs from 19 independent transformants were sequenced by using primers near the *nusA11* allele. All but two of the plasmid DNAs contained an A-to-G change at position 542, a true reversion of *nusA11*. Of the two, one had an A-to-C change at the *nusA11* site, creating alanine 181 (*nusA1101*; Fig. 1); the other retained the *nusA11* substitution. Further sequence studies revealed that this plasmid carried a substitution of T for A at base position 250, changing aspartate 84 to tyrosine (*nusA1102*; Fig. 1), implying putative interaction between aspartate 84 and glycine 181.

We have described the isolation and characterization of murine monoclonal antibodies against NusA and have shown that glycine 181 and leucine 183 constitute part of the epitope for the N14 antibody (39). N14 binds to the wild-type NusA protein but not to the *nusA11* or *nusA1* mutant protein. We tested the antigenic reactivities of the true and pseudorevertant NusA proteins to N14 monoclonal antibody. Plasmids encoding *nusA*$^+$, *nusA1*, *nusA11*, *nusA1101*, and *nusA1102* were transformed into the host strain AT1103, which carries the amber *nusA* mutation. The *nusA134* mutant produces a truncated NusA protein of two-thirds normal length which is distinguishable from the plasmid-encoded NusA (51). Since truncated NusA is active at 32°C in the absence of the amber suppressor (51), these transformant cells were grown at 32°C in the absence of amber suppressor.

As shown in Fig. 4, rabbit polyclonal antibodies reacted with all *nusA* products, namely, from *nusA*$^+$, *nusA1*, *nusA11*, *nusA1101*, *nusA11 nusA1102*, and *nusA134* strains, whereas the N14 antibody reacted with the *nusA*$^+$, *nusA11* true revertant, and *nusA134* products, but not with the *nusA1101* and *nusA11 nusA1102* or the *nusA1* and *nusA11* altered proteins. These results demonstrated that the aspartate-to-alanine change in *nusA1101* restored the function of NusA without restoring the epitope structure for N14.

Intergenic *nusA11* suppressor mutation in the *rpoC* gene.

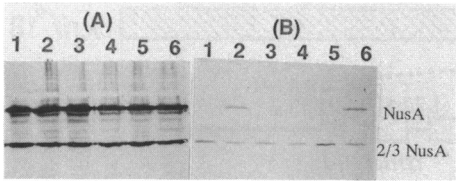

FIG. 4. Immunoblotting analysis of mutant *nusA* proteins. AT1103 cells (*nusA134*) carrying plasmid pMS1 and its derivatives were grown at 32°C, and their total proteins were subjected to electrophoresis and immunoblotting. (A) Rabbit polyclonal antibody; (B) murine monoclonal antibody N14. The *nusA* alleles encoded in the plasmids are: lane 1, *nusA1*; lane 2, true reversion of *nusA11*; lane 3, *nusA11 nusA1102*; lane 4, *nusA1101*; lane 5, *nusA11*; lane 6, *nusA*$^+$.

Temperature-resistant colonies of *nusA11* mutant cells (YN2351) were selected at 42°C. YN3428 was one of those revertants which contained an intergenic suppressor mutation, named *sna* for suppressor of *nusA*. P1 phage grown on the YN2345 strain, carrying a Tn*10* transposon (*wxz*::Tn*10*) cotransducible with *nusA*, was used to transduce YN3428 to tetracycline resistance. Five randomly selected transductants were then used as donors in back-cross P1 transduction to the wild-type strain R594. P1 lysates from three transductants were able to cotransduce temperature-sensitive lethality with the Tn*10* marker at ~60% frequency. Therefore, it was concluded that the YN3428 revertant strain retained the *nusA11* allele.

A mutation, *sna-10*, which restored growth of *nusA11* mutant cells was mapped at the 90-min region of the chromosome by conjugation with an Hfr strain (KY1397) which transfers the chromosome in a clockwise direction starting from the *ilv* region (data not shown). A more precise location was determined by P1 transduction. P1 grown on the YN2572 strain, carrying a Tn*10* transposon (*wzy*::Tn*10*) with cotransducible rifampin sensitivity (Rif^s), was used to transduce RM24 (*nusA11 sna-10* Rif^r) to tetracycline resistance (Table 4). The data from three-point crosses revealed that *sna-10* maps between Rif^r and Tn*10*, being 92.6% cotransducible with Rif^r. As a rifampin-resistant mutation affects *rpoB*, encoding the β subunit of RNA polymerase, and the Tn*10* transposon used is located clockwise to Rif^r on the map, the above results suggested that the *sna-10* allele maps nearby but downstream of *rpoB*. A reciprocal cross was conducted with YN2634 (*sna-10 wzy*:Tn*10*) as the donor and RM62 (*nusA11*) as the recipient. Eight of 10 tetracycline-resistant transductants grew at 42°C. The occurrence of *nusA11* in the temperature-resistant colonies was confirmed by back-cross P1 transduction. Therefore, the mutation in the *rpoBC* region was sufficient to suppress *nusA11*.

The linkage of *sna-10* and Rif^r suggested that *sna-10* may be in the *rpoC* gene, encoding the β′ subunit of RNA polymerase. This possibility was examined by complementation tests. λ *rif*^{d}18 transducing phage, isolated by Kirschbaum and Konrad (26), carries a ~26-kb chromosomal DNA segment including *rpoB* and *rpoC*. The *nusA11 sna-10* cells lysogenic for λ *papa* (RM37) were infected with λ *rif*^{d}18 (encoding the heat-sensitive λ repressor *c*I857 and dominant Rif^r), and rifampin-resistant colonies were selected at 32°C as λ *rif*^{d}18 lysogens. These colonies failed to grow at 42°C. However, there appeared to be two distinct classes: one grew at 40°C (leaky temperature sensitivity), whereas the other failed to grow at 40°C (tight temperature sensitivity).

Phage lysates were prepared from these colonies and tested for transduction of the *rif*^{d} marker. Lysates from the former leaky group transduced cells to rifampin resistance at high frequency, whereas those from the latter group were unable to transduce cells to Rif^r (data not shown). These results were interpreted as indicating that the former group carried λ *rif*^{d}18 prophage which was integrated by homologous recombination via a single reciprocal crossover between λ *rif*^{d}18 and the chromosome, and the latter group had lost the transducing phage, presumably after a double reciprocal crossover between the homologous regions of λ *rif*^{d}18 and the bacterial chromosome, giving rise to a *rif*^{d} bacterial recombinant. In the λ *rif*^{d}18 lysogen, *sna-10* was recessive to the wild-type allele on the phage, though the *sna-10*/*sna*$^+$ heterodiploid slightly suppressed *nusA11* at intermediate temperatures.

The bacterial segments carrying *rplL-rpoB-rpoC* and *rplL-rpoB* were subcloned from λ *rif*^{d}18 DNA into plasmid pSU2719, giving rise to pSU-LBC^W and pSU-LB^W, respectively; plasmid pSU-LBC^W was further manipulated to make *rpoB*- and *rpoC*-disrupted plasmids pSU-L(B::Kan)C^W and pSU-LB(C::Kan)^W, respectively (Fig. 5). RM65 (*nusA11 sna-10*) cells were transformed at 32°C with these plasmid DNAs and scored for growth at 42°C. As shown in Table 5, pSU-LBC^W and pSU-L(B::Kan)C^W restored temperature-sensitive growth, while pSU-LB^W and pSU-LB(C::Kan)^W did not. Conversely, the pSU-LBC^M plasmid, encoding the *sna-10* allele, allowed the *nusA11* mutant cells (RM62) to grow at 42°C upon transformation, while pSU-LB^M, which deleted *rpoC*, did not. These results led us to conclude that the *sna-10* mutation is in the *rpoC* gene and that a higher copy number of the *sna-10* allele could dominate the wild-type allele, unlike in the diploid condition. The marker rescue experiment further proved the conclusion that plas-

TABLE 4. Tranductional mapping of the *sna-10* mutation[a]

Donor (relevant markers)	Recipient (relevant markers)	Selected marker	Distribution of unselected markers		
			Class	No.	Frequency (%)
YN2572 (Tet^r *sna*$^+$ Rif^s)	RM24 (Tet^s *sna-10* Rif^r *nusA11*)	Tet^r	*sna*$^+$ Rif^s	291	*sna*$^+$ Tet^r, 89.7
			sna$^+$ Rif^r	22	
			sna-10 Rif^s	4	Rif^s Tet^r, 84.5
			sna-10 Rif^r	32	
YN2634 (Tet^r *sna-10 nusA11*)	RM62 (Tet^s *sna*$^+$ *nusA11*)	Tet^r	*sna-10*	8	*sna-10* Tet^r, 80
			sna$^+$	2	

[a] Recipient cells were infected with phage P1 *vir* grown on donor cells, incubated for 20 min for adsorption, further incubated in L broth (34) at 32°C for 30 min, and plated on L agar containing 15 μg of tetracycline per ml for Tet^r selection. After incubation for 2 days at 32°C, colonies were picked, purified, and scored for unselected markers. Temperature-sensitive growth was scored on YT agar plates, and rifampin sensitivity was tested on L agar containing 50 μg of rifampin per ml.

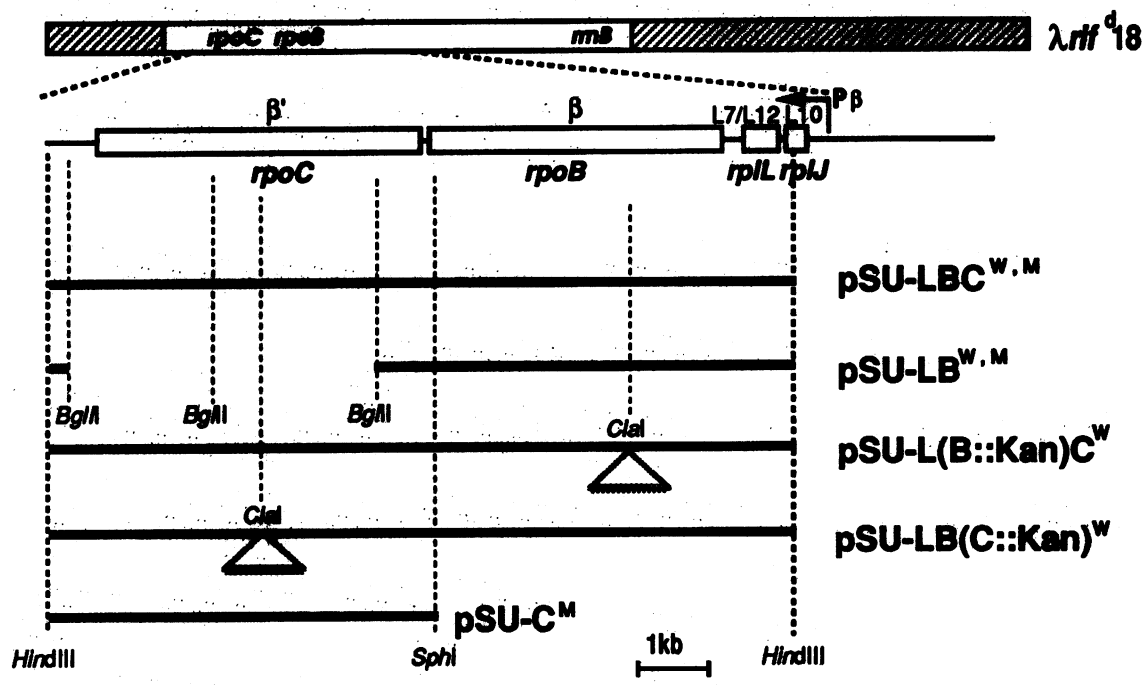

FIG. 5. Structure of the *rpoBC* region and DNA segments cloned on phage and plasmids. The hatched region of λ *rif*^{d}18 represent λ DNA, and the open region is bacterial DNA. A transcription unit is indicated as P (promoter) and a horizontal arrow showing the direction of transcription. Heavy solid lines represent regions of bacterial DNA cloned in the plasmids. Triangles represent insertions of the Kan^r gene. Only relevant restriction sites are included.

mid pSU-C^M, which carries the mutant *rpoC* gene but does not express it, enabled the *nusA11* strain to generate temperature-resistant colonies at high frequencies by recombination (Table 5 and Fig. 5).

Increased transcription termination in the altered β' subunit mutant. It has been shown that *nusA11* causes leaky termination at the λ t_{R1} terminator (37). To investigate the effects of the *sna-10* mutation on termination, the frequency of t_{R1} termination was examined. Plasmid pMZ105 has a λ DNA fragment insert of 400 bp which encodes *boxA*, *nutR*, and t_{R1} signals between the *gal* promoter and the *galK* gene in plasmid pKG100. Cells of R594 (*galK nusA*$^+$ *sna*$^+$), RM63 (*galK nusA*$^+$ *sna-10*), RM62 (*galK nusA11 sna*$^+$), and RM65 (*galK nusA11 sna-10*) were transformed with these plasmid DNAs, and galactokinase activities were measured at 32 or 42°C (Table 6). Plasmid pKG100, a control plasmid lacking t_{R1}, gave similar galactokinase activities at 32 and 42°C, though a small reduction in the galactokinase level was observed in the *nusA11* strains (RM62 and RM65) at 42°C. The values observed in the *sna*$^+$ and *sna-10* strains carrying pMZ105 indicated that t_{R1} termination is 87% efficient in the wild type at 42°C and increased to 94% in the single *sna-10* mutant. Efficiencies of termination in *nusA1* and *nusA1 sna-10* doubly mutant cells coincided with those in *nusA*$^+$ and *nusA*$^+$ *sna-10* cells, respectively. The reduction of t_{R1} termination in *nusA11* cells was counteracted by *sna-10*. These results clearly demonstrated that the altered β' subunit resulting from the *sna-10* mutation conferred increased termination capacity on RNA polymerase and led to suppression of *nusA11*.

TABLE 5. Complementation of the *sna-10* mutation[a]

Strain (relevant genotype)	Plasmid or phage	Growth at 42°C[b]
RM37 (*nusA11 sna-10* [λ *papa*])	λ *rif*^{d}18	−
RM65 (*nusA11 sna-10*)	pSU2719	+
	pSU-LBC^W	−
	pSU-LB^W	+
	pSU-L(B::Kan)C^W	−
	pSU-LB(C::Kan)^W	+
RM62 (*nusA11 sna*$^+$)	pSU-LBC^M	+
	pSU-LB^M	−
	pSU-C^M	−*

[a] Phage or plasmids were transduced into the mutant cells by transformation or infection; selection was made for antibiotic resistance at 32°C (rifampin resistance for λ *papa* and chloramphenicol resistance for the plasmids). These transformants and transductants were purified and scored for growth at 42°C on YT agar plates containing the selective antibiotic.

[b] Symbols: +, growth; −, no growth; −*, no growth but appearance of temperature-resistant colonies at high frequency (~10^{-4}).

It is known that the *nusA10* mutation causes leaky termination at the *rrnB* T1 terminator of *E. coli* (22). The efficiency of t_{R1} termination in RM35 (*nusA10*) cells was reduced to 66% at 42°C, and *sna-10* increased the termination frequency to 95% (Table 7). However, the *nusA10 sna-10* doubly mutant cells (RM38) failed to grow at 25°C. Therefore, suppression of growth by *sna-10* was specific to the *nusA11* allele, though reduced t_{R1} termination in *nusA10* and *nusA11* cells was restored by *sna-10*. Several revertant colonies which grew at 25°C were isolated from RM35 (*nusA10*). The frequency of t_{R1} termination in these revertants was not correlated with the growth phenotype (Table 7). These observations were consistent with the failure of *sna-10* to suppress growth of the *nusA10* strain and suggested that a defect in termination at rho-dependent terminators such as λ t_{R1} is not a cause of the cold-sensitive lethality of *nusA10*. None of the cold-resistant colonies

TABLE 6. Effect of *sna-10* on termination frequency at the λ t_{R1} terminator

Strain (relevant genotype)	Plasmid	Galactokinase activity[a] (U)		Activity ratio, pMZ105/pKG100[b]	
		32°C	42°C	32°C	42°C
R594 (*nusA*$^+$ *sna*$^+$)	pKG100	359	467	0.15	0.13
	pMZ105	54	59		
RM63 (*nusA*$^+$ *sna-10*)	pKG100	323	511	0.07	0.06
	pMZ105	24	28		
RM62 (*nusA11 sna*$^+$)	pKG100	355	239	0.50	0.53
	pMZ105	177	127		
RM65 (*nusA11 sna-10*)	pKG100	330	233	0.26	0.16
	pMZ105	85	37		
RM61 (*nusA1 sna*$^+$)	pKG100	ND[c]	500	ND	0.13
	pMZ105	ND	63		
RM64 (*nusA1 sna-10*)	pKG100	ND	514	ND	0.04
	pMZ105	ND	21		

[a] Exponentially growing cultures at 32°C and those exposed to 42°C for 1 h were measured for galactokinase activities as described in Materials and Methods. Background galactokinase activities were <1 U in strains carrying the control plasmid pBR322. See Table 7, footnote *a*, for definition of units.
[b] Ratio of galactokinase activity units from plasmids pMZ105 and pKG100, which represents efficiency of termination.
[c] ND, Not determined.

Rev-1 through Rev-7 contained true reversions of either *nusA10-1* or *nusA10-2* (data not shown).

DISCUSSION

The peptide sequence of NusA protein from amino acid position 167 to position 191 was corrected by nucleotide sequence, gene fusion, and peptide sequence analyses. The same nucleotide corrections were made by Craven and Friedman (4a). In fact, the occurrence of T at base position 500 generates a methionine residue at position 167 which is susceptible to cyanogen bromide cleavage. Thus, cyanogen bromide digestion led us to read the peptide sequence LPRENFRPGDRVRGVLYS from position 168 to position 185. The 164 to 191 peptide region is hydrophilic, rich in arginine and acidic residues (7 and 5, respectively, of 28 residues). The arginine-rich motif has been found in several RNA-binding proteins, some of which are antitermination proteins, including N of phages λ, 21, and 22 and Nun of phage HK022 (30). The arginine-rich motif of these N and Nun proteins is thought to play a crucial role in specific recognition of the *boxB* RNA hairpin. However, the arginine-rich sequence of NusA does not coincide with the consensus in N or Nun. Therefore, we assume that the arginine-rich domain of NusA is involved in nonspecific binding to RNA or binding to a specific RNA element other than the *boxB* hairpin.

It is tempting to imagine a specific interaction between this arginine-rich domain of NusA and the *boxA* sequence. Previous genetic and biochemical experiments have implicated interaction between NusA and *boxA* RNA and/or the RNA of a region close to *boxA* (10, 50). The N14 monoclonal antibody, which specifically recognizes glycine 181 and leucine 183, does not inhibit NusA binding to RNA polymerase even in large excess (39). Therefore, the *nusA11* and *nusA1* substitutions may not affect polymerase-NusA interaction but rather RNA-NusA interaction. The *nusA1* mutation substituted arginine for leucine 183, shifting the region to be more arginine rich, while the *nusA11* mutation replaced glycine 181 with aspartate, which changes the charge. Distinct phenotypes of these mutants, a defect in N antitermination and λ growth by *nusA1* and a defect in termination and *E. coli* growth by *nusA11*, may be attributed to the opposite effect on RNA binding strength or to an altered binding specificity. The *nusA1* substitution effect may be explained by assuming that the mutant NusA protein binds improperly to RNA because of its increased or altered RNA-binding activity and interferes with the formation of functional antitermination complex (37, 38).

The occurrence of the reversion mutation *nusA1101*, which replaced alanine for the mutated aspartate 181, indicated a neutral amino acid requirement at position 181 for proper functioning of NusA. The occurrence of the intragenic *nusA11* suppressor mutation *nusA1102*, which replaced tyrosine for aspartate 84, suggested that aspartate 84 is located close to glycine 181 in the tertiary structure and that the loss of an acidic charge in this environment leads to suppression of the *nusA11* mutation.

Most of the catalytic activities required for RNA synthesis, i.e., substrate binding, phosphodiester bond formation, and antibiotic binding, are localized to the β subunit of RNA polymerase (for reviews, see references 20 and 54). On the other hand, the role of the β′ subunit is little understood. The only inference as to the biochemical feature of β′ was a nonspecific DNA-binding capacity owing to the protein's positive charge (56). Temperature-sensitive lethal *rpoC* mutations have been isolated and prove that the β′ subunit is essential for cell growth (43). Some of the β′ mutations cause defects in the assembly of RNA polymerase (49).

In this study, we found that the *sna-10* mutation, isolated as a suppressor of the temperature-sensitive lethal *nusA11* mutation, is in the *rpoC* gene. The *sna-10* mutation per se conferred increased termination of transcription at t_{R1}, indicating that the β′ subunit of RNA polymerase is involved in transcription termination. Consistent with this finding, Jin and Gross have suggested that the mutation *rpo-214*, isolated as a suppressor of the *rho-201* mutation (17), is located in the *rpoC* gene by the marker rescue test (23). Thus, we infer that the β′ subunit participates in transcription termination in *E. coli*. The *sna-10* mutation will be referred to as *rpoC10*.

The occurrence of a suppressor mutation of *nusA11* in *rpoC* indicates a genetic interaction between the arginine-

TABLE 7. λ t_{R1} termination in the cold-sensitive *nusA10* mutant and its derivatives[a]

Strain (relevant genotype)	Galactokinase activity[a] (U)		Activity ratio, pMZ105/pKG100
	pKG100	pMZ105	
R594 (*nusA*$^+$ *sna*$^+$)	518	76	0.15
RM35 (*nusA10 sna*$^+$)	666	232	0.34
RM38 (*nusA10 sna-10*)	366	19	0.05
RM35 revertants			
Rev-1	448	235	0.52
Rev-2	202	33	0.16
Rev-3	493	125	0.25
Rev-4	555	65	0.12
Rev-5	565	87	0.15
Rev-6	535	353	0.66
Rev-7	649	275	0.42

[a] See Materials and Methods for details. Cultures were grown at 42°C. Galactokinase activities are expressed as nanomoles of galactose phosphorylated per minute per A_{650} unit. pMZ105/pKG100 indicates ratios of galactokinase units from plasmids pMZ105 and pKG100. Revertant colonies of RM35 grew at 25°C. Background levels of galactokinase in these strains were <1 U.

rich domain of NusA and the β′ subunit of RNA polymerase. However, this may not be explained by their direct interaction, because the arginine domain-targeting antibody N14 is unable to inhibit NusA-RNA polymerase binding. We infer that the *nusA11* suppressor mutation in *rpoC* may be explained by assuming that *sna-10* indirectly suppresses *nusA11* by altering termination by RNA polymerase, perhaps by a changed interaction with RNA at the termination site or with Rho protein. Further genetic and biochemical analyses of the *nusA* and *rpoC* mutations are needed.

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